BINUS UNIVERSITY

MONOGRAPH

SMOKESCREEN GENOTYPING ARRAY (GTA) INDONESIA

BENS PARDAMEAN

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PREFACE

Smoking is a huge problem in Indonesia and throughout the world. Smoking negatively impacts every system in the human body causes many types of cancer. Even when smokers want to quit, only 4-7% are successful. Researchers are developing approaches to improve success rates, including using genetics to guide treatment. Sponsored by the USA National Institutes of Health (NIH), Dr. Baurley and colleagues developed the most advanced screening technology available for understanding the genetics of addiction. This technology is being used by scientists to discover the optimal smoking cessation program for smoker's genetics.

To use the Smokescreen technology, a DNA sample is extracted in a lab from a sample of saliva or blood. DNA samples are placed on the Smokescreen Genotyping Array (GTA), which is read by a high throughput machine, examining over 500,000 genetic markers in parallel. The raw data is then processed in a workflow to call the genotypes for each individual. The data is thoroughly quality controlled and available for analysis and interpretation by a scientist.

TABLE OF CONTENT

Synopsis

Genetics influence nicotine metabolism, smoking behavior, smoking-related diseases, and response to treatment differently by population. As such, Indonesian smokers will have unique characteristics that influence these phenotypes. Bina Nusantara (BINUS) University invested in two critical areas of research to help Indonesian smokers and victims of tobacco-related diseases.

The first aim was to assess the awareness of physicians and smokers to smoking cessation treatment approaches and the possible benefits of genetic testing.

The second aim was to perform a highly innovative research study in Indonesia with the Smokescreen technology. The study would be the first of its kind in Indonesia and provide the initial data for scientists to develop biosignatures of smoking cessation and cancer in Indonesia.

Chapter 1
Study Collaborators

Bina Nusantara University contributed one plate of Smokescreen arrays (96 DNA samples), supported the BDSRC team, and provided high performance computing resources. Dr. Bens Pardamean and Dr. James Baurley were co-investigators at this site and focused on project management, data management and analysis aspects of the study.

Hasanuddin University contributed one plate of Smokescreen arrays (96 DNA samples), and supported participant recruitment, data, and DNA sample collection and storage. Co-investigators included dr. Upik Miskad, Prof. Dr. Irawan Yusuf, dr. Ronald Lusikooy, and Prof. Dr. Andi Fachruddin.

BioRealm contributed the Smokescreen toolkit and provided biostatistics and statistical genetics support. The BioRealm team consisted of Dr. James Baurley, Dr. Carolyn Ervin, Dr. Andrew Bergen, Chris Edlund, Stephen McGee, and Carissa Pardamean.

NVIDIA provided GPGPUs and training to the BDSRC for the analysis of the genomic data. Dr. Ettikan Karuppiah was a key person in providing these resources to the study.

RUCDR Infinite Biologics handled the sample processing and genotyping for the DNA samples sent to the USA. Dr. Andy Brooks, Dr. Jay Tischfield, and Dana Witt-Garbolino provided support to ensure high quality data production.

The University of Southern California (Dr. Jane Figueiredo) was our academic partner on the topics of epidemiology and global health.

Clemson University (Dr. Chris Mcmahan) is our academic partner in the biomathematical aspects and potential novel statistical methodologies for analysis of the data.

Chapter 2
Smokescreen Genotyping Array (GTA)

Smokescreen GTA is a unified platform for genetic research on smoking behavior, addiction, pharmacological treatment, and related disease. Smokescreen GTA includes 646,247 markers in 23 categories. The array design covers genomewide common variation (66%, 82%, and 91% in African (YRI), East Asian (ASN), and European (EUR) respectively); most of the variation with a minor allele frequency ≥ 0.01 in 1,014 addiction genes (85%, 90%, and 90% for YRI, ASN, and EUR respectively); and, all variation in the known regions related to smoking behavior (CHRNA5-CHRNA3-CHRNB4) and nicotine metabolism (CYP2A6- CYP2B6). Under contract HHSN271201300004C (PI:Baurley) and DA033813 (PI:Bergen) funding, 346 study samples, 187 HapMap (http://hapmap.ncbi.nlm.nih.gov/), and 1000 Genomes Project (1KGP, http://www.1000genomes.org/) samples were selected for Smokescreen GTA genotyping.

Chapter 3
Market Research: Physicians

3.1 Introduction

The act of smoking is deeply ingrained in the Indonesian people's daily lives and social culture. Indonesia is the fourth highest cigarette consumer in the world after China, Russia, and USA1. As of 2011, approximately 60 million people in Indonesia smoke cigarettes on a daily basis with an average daily cigarette consumption of 12-13 sticks[2]. Indonesia is one of the few countries in the world with much higher rates of smoking in males (57%) than females (under 4%)[1]. Smoking initiation typically begins around the ages of 17-18 with almost 13% of smokers beginning before the age of 15[2].

Smoking cessation remains to be the most effective way to reduce the impacts of tobacco consumption. The effects of smoking cessation materialize much more rapidly than the damaging effects of smoking. Additionally, individuals who began smoking at an early age yet stopped smoking before the age of 40 has a 90% higher likelihood of avoiding tobacco-attributed diseases[3]. A recent survey of Indonesian smokers discovered relatively low interest in quitting (only 48% compared to 70% for countries such as United States and United Kingdom)[4, 5]. The small percentage of Indonesian smokers who did quit, achieved it through no aid whatsoever, which further contributes to the difficulty of smoking cessation in the nation2.

A vital basis for low success rates in quitting smoking without aid is nicotine's stronghold on brain physiology. Pharmacotherapy is the most effective method to deal with withdrawal symptoms caused by neurochemical responses to nicotine absence, thereby increasing the chance for long-term cessation by 50-70%. This is particularly true for smokers who consume between 10-15 cigarettes per day[6]. A well-established biological basis to nicotine dependence is found in genetics, particularly in variations of the hepatic cytochrome P450 2A6 (CYP2A6) and nicotinic receptors (CHRNA) genes. The CHRNA gene family is involved in smoking-behavior patterns (e.g. smoking heaviness, time to first cigarette per day) through its relation to the neurological reward system from nicotine intake[7]. Nicotine metabolism and clearance by the liver is heavily influenced by CYP2A6 gene variation; these variants could also be useful in determining an individual's likelihood in lung cancer development due to smoking[8]. Moreover, genes relevant to nicotine

dependence and metabolism have been found to be essential in determining the efficacy of different pharmacotherapy methods as well as in predicting risk of smoking relapse[9].

Genetic screening and notification have the potential to advance patient treatment[10]. Since genetic variation impacts nicotine metabolism, smoking behavior, and a cessation method's efficacy, it becomes a significant puzzle in research and for physicians to recommend the best approach for a patient wanting to quit. Within the context of smoking cessation, genetic screening information has been shown to increase cessation success rate and improve cessation interest through heightened patient health awareness[11]. Several FDA-approved drugs in the United States are available for smoking cessation such as varenicline and bupropion. However, physicians are cautious about prescribing them due to potential adverse, psychiatric side effects. The nicotine metabolite ratio (NMR), which is heavily influenced by CYP2A6 gene variants, has shown promise as a biomarker to optimize pharmacotherapy assignments by improving efficacy and reducing side effects. From a medical practice standpoint, this means that physicians could be assigning nicotine dependence treatments to their patients based on a blood test to assess their NMR profile in the near future11. While these findings are particularly encouraging, there are barriers to using the NMR in practice. Physicians may still misunderstand available treatment approaches, genetic screening's role and limitation, and proper ways to interpret and deliver results to patients[12].

Policy-based interventions in tobacco-product use in Indonesia are limited, largely non-governmental supported, and are subject to legal interferences by tobacco companies[13]. A strong symbol of this is Indonesia's status as the only Southeast Asian nation yet to sign the WHO Framework Convention on Tobacco Control Treaty[2], which implementation has proven to be an effective way of reducing tobacco consumption[3, 13]. Several investigations that are part of the Quit Tobacco International Project based in the province of Yogyakarta find that 75% of the physicians have a lax attitude about asking patients about their smoking habits. 20% of the surveyed physicians (particularly male) are smokers themselves while 80% of the physicians believe that smoking up to 10 cigarettes per day does not harm a person's health[2, 14]. However, these studies tend to be in a single site and have comparatively small samples. Given that the health care providers are a part of the nation-wide public health problem, it is important to assess their interest in improving care for smoking cessation and smoking-related diseases at multiple cities and institutions. One tool that is under-utilized in Indonesia is medical

genetics, which ties in strongly with pharmacotherapy and its selection to improve efficacy through physician advise.

The appropriate pharmacotherapy cessation aid can significantly impact an individual's success at quitting smoking, thereby placing a vital role on physicians. Due to the lack of official reports on genetic screening interest for smoking cessation in Indonesia, this study first investigates physicians' interest in using genetic-based screening within the context of smoking cessation consultation. The study also looks at the Indonesian health care providers' interest in information on smoking-related diseases as well as physicians' current access to medical genetics for their medical practices.

3.2 Materials and Methods

This study was funded by Bina Nusantara University to evaluate physician interest in genetic technologies and screening programs. Questionnaires were the primary data collection instrument. The physician questionnaire consisted of 12 questions, surveying participants on: medical specialty, health care facility affiliation, patient population profile, medical research interest, opinion on genetic screening technology, and medical genetics tools accessibility.

Purposive sampling led to the participation of physicians from several urban cities across Indonesia, including the greater Jakarta metropolitan area, Bandung (West Java), Semarang (Central Java), Yogyakarta, Malang (East Java), Palembang (South Sumatra), and Makassar (South Sulawesi). All 113 health care professionals responded to a hard-copy questionnaire in-person and completed the questionnaire at the site of their health care facility posts during their own time. The survey targeted physicians with interests in respiratory diseases, nicotine addiction, and cancer pathology since they were assumed to have higher interest in smoking cessation and smoking-related diseases. The responses were entered into a MySQL database by trained data entry staff[15]. All statistical analyses were performed on [R], particularly Pearson's chi-squared test or Fisher's exact test to quantify associations between opinions of genetic screening usefulness and physicians' profile[16]. Associations with usefulness were tested for each profile category (organization type, medical specialty, research interest). For organization type and research interest, each individual item's association with usefulness was assessed as well. P-values less than 0.05 were interpreted as statistically significant associations.

3.3 Results

113 surveys were collected and Table 1 summarizes the physician survey's results. Most of the physicians surveyed were affiliated with a clinic (56%). 63% of the physicians were general practitioners, reflecting the predominant affiliation to clinics. The most dominant research interests were respiratory diseases (35%) and nicotine addiction (28%).

Overall, 81% (n = 89) of total respondents indicated that genetic screening information for smoking cessation would be useful. Institutional affiliation and usefulness have a statistically significant association (p = 0.018). Individual categories for research interest were tested only independently against usefulness since a physician could select more than one research interest. The usefulness rating was significantly associated for interest in cancer pathology (p = 0.005) and in other research fields (grouping among 13 categories written-in by the physicians; p = 0.003). Usefulness was not significantly associated with interest

Table 1. Profile of surveyed physicians - Genetic Screening Usefulness for Smoking Cessation

Category	Total N (%)	Very Useful	Somewhat	Not	p-value
Institutional Affiliation					0.018
Hospital	48 (44)	21 (45)	22 (48)	3 (7)	0.043
Clinic	62 (56)	16 (26)	30 (49)	15	0.108
Medical Specialty					0.222
General Practitioner	71 (63)	19 (28)	38 (55)	12	
Non-GP	42 (37)	18 (44)	17 (41)	6 (15)	
Medical Research					
Respiratory Diseases	40 (35)	17 (47)	12 (33)	7 (19)	0.060
Cardiovascular Diseases	19 (17)	8 (42)	6 (32)	5 (26)	0.423
Cancer Pathology	9 (8)	7 (78)	2 (12)	0 (0)	0.005
Nicotine Addiction	32 (28)	15 (47)	15 (47)	2 (6)	0.074
Substance	7 (6)	1 (14)	5 (72)	1 (14)	0.423
Others	34 (30)	5 (15)	20 (59)	9 (26)	0.003

in respiratory disease and nicotine addiction (p = 0.06 and 0.074 respectively). Medical specialty was not significantly associated with usefulness ratings (p = 0.222).

Table 2 summarizes the physicians' expected and current experience with genetic screening. The survey found that 42% of physicians were interested in genetic information on respiratory, cardiac, and psychiatric diseases. 23% were interested in genetic information on addiction. 42% of

physicians believed that they did not have enough knowledge to decide what type of information from genetic screening would be useful. Nonetheless, 70% of those who deemed themselves to lack knowledge stated that genetic screening information would be useful at some level for smoking cessation. 89% of physicians stated that their affiliated institutions were limited in medical genetics services and facilities.

The survey also included a question that asked physicians to report the percentage proportion of their patients who are current smokers. 27% (N = 30) of the surveyed physicians could not provide a response to this question. Based on the physicians who did respond to the question (N = 83; 73%), it was found that smokers, on average, comprise 45% of a physician's patient list.

Table 2. Indonesian physician and genetic screening

Existing Medical Genetics and Related Services (n = 113)	n	%
Genotyping	9	8
Sequencing	3	3
None - institution limited in this area	101	89
Genetic Screening Information Interest (n = 113)		
Genome-wide analysis	9	8
Addiction-related gene screening	26	23
Pharmacogenetics	22	19
Respiratory, cardiac, psychiatric diseases screening	48	42
I don't know - better leave decision to experts	47	42

3.4 Discussion

A 2011 survey performed by the World Health Organization (WHO) on Indonesian smokers finds that only two-thirds of the surveyed patients recalled ever having a physician ask them about their smoking status [2, 14]. Our study complemented this finding with 27% of the surveyed physicians not knowing how many smokers they were currently treating. The lack of knowledge on patient smoking status is worrisome since proactive health care providers play a crucial role in smoking cessation success. Physicians are recommended to practice the 5 A's in smoking cessation counseling: Ask, Advise, Assess, Arrange, and (re-)Assess[17].

It is also important for physicians to understand how to interpret genetic screening results and effectively relay the findings to patients[18]. This is especially true given major concerns that primary care physicians and general practitioners have over the sensitivity and complexity of medical genetics information[19].

Despite the relatively low interest in patient smoking status and limited resources for genetic screening available to Indonesian physicians, we found a positive response towards medical genetics for smoking cessation. Even 70% of those who did not know what type of information they would like from genetic screening stated that having it as a medical practice tool would be useful for at least smoking cessation. This is promising because 56% of the physicians surveyed were based in clinics. Compulsory community clinics remain the most affordable and most widely-visited form of health care for the Indonesian population, making these clinics the most far-reaching source of information on smoking cessation and smoking-related diseases[20].

One setback in the survey collection was the oversight of not interviewing the physicians in-person. The logic to this was to respect the privacy of the physicians by having them complete the questionnaires at their own time. However, this led to some missing values to the questionnaires. The survey also did not have a direct question to gauge how comfortable physicians would be in implementing or incorporating genetic screening information into their daily medical practices if the tools became available to them, taking upon the role of a provider and interpreter of medical genetics information.

Physician opinion is one side of the story when it comes to smoking cessation efforts on a public health scale. Opinions and attitudes of patients are also crucial pieces of the puzzle. Therefore, the next step to our research is to survey smokers' opinion on genetic screening for smoking cessation.

We observed an optimistic and promising interest in genetic screening among Indonesian physicians for smoking cessation and related diseases despite limitations in the availability of medical genetics facilities. Most physicians who stated that they lack the proper knowledge to specify information they wish to gain from genetic screening still believe that the tool would be useful for smoking cessation consultation and nicotine dependence treatment. Therefore, education on information use and interpretation, facility and technology availability, and most likely monetary costs would be barriers in efforts to implement genetic screening for smoking cessation consultation in Indonesia.

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Chapter 4
Market Research: Smokers

4.1 Introduction

Smoking is a leading cause of preventable death in Indonesia and around the world. Yet, 1 in 3 males in Indonesia smoke because of the highly addictive properties of nicotine and complex behavioral and social factors. Genetic factors play a very significant role in how a smoker metabolizes nicotine, how dependent they are, and how they respond to treatment. Given the number of treatments available, both pharmacotherapies and behavioral, the best smoking cessation path varies from individual to individual. Optimizing the path for smokers wanting to quit will reduce smoking rates and reduce the expenses related to healthcare and lost productivity due to smoking. Research on which genetic factors are important to smoking cessation success and how best to translate these findings into real-world applications is ongoing.

Recognizing the need for improving the understanding of the genomics of addiction and developing screening for personalized smoking cessation approaches, we developed a state-of-the-art genetic screening platform called Smokescreen (Baurley, et al., 2016). The technology captures genetic markers of nicotine dependence and treatment approaches, and to addiction in general. The technology includes hardware for capturing raw data and software for analyzing and interpreting the data. The platform works by a DNA sample (extracted from saliva or blood of smokers) being sent to a lab set up to use the technology. The sample is processed on a custom assay. The genomic data is then cleaning and analyzing and then results are returned. The Smokescreen process is being heavily used in nicotine and tobacco research in the United States and is beginning to be used in research within Indonesia.

The evidence is accumulating for a panel of genetic markers, whose results could be used to guide smoking cessation therapies for smokers wanting to quit. The value of screening for optimized smoking cessation treatment is well known in the US. It is estimated that even an improvement in smoking cessation success rates of 1% would represent an annual savings of US$ 3 billion in expenses related to health-care and lost productivity. There is a large push for translation of research to smoking cessation application in the U.S. The market barriers in the U.S. include

government regulation for diagnostic testing as well as acceptance of new pharmacotherapies and genetic screening by smokers and physicians. The smoker must want to quit and embrace the provided plan and the physician must accept the technology and support its guidance.

There is interest in the marketability of genetic screening technologies in Indonesia, a country highly impacted by smoking and attributable disease. For an initial assessment of the market, we surveyed the end-user of new technologies for smoking cessation – current smokers in Jakarta, Indonesia. With this initial assessment, we can naturally extend it to physicians that assist smokers in quitting, and more largely the pharmaceutical companies and health-care networks that would significantly benefit from improved smoking cessation treatment and reduced smoking rates in Indonesia.

In this report, we present results from a market survey designed to characterize smokers, assess availability of cessation treatments and screening methods, and identify barriers and opportunities for genetic screening technologies. Motivated by the health impact of smoking, the questions were designed to help characterize smokers in Indonesia, particularly in their experiences towards smoking cessation attempts and methods, as well as in their opinions towards factors influencing smoking cessation and medical genetics. This will provide an initial market assessment for genetic screening for optimal smoking cessation treatment in Jakarta. Additionally, this work will help capture the requirements for developing and customizing products and services related to Smokescreen technology used in the U.S. for Indonesian populations.

The tobacco industry has a significant and active role in Indonesia due to the country's lax regulations on tobacco product advertisement and its low tobacco taxation [17, 15]. Aside from non-stringent government regulations and the aggressive marketing by tobacco companies, the act of smoking is deeply ingrained in the Indonesian people's daily lives and social culture. Indonesia is the fourth highest cigarette consumer in the world, after China, Russia, and the US [34]. The lack of excise tax regulations on tobacco products leads to low cigarette prices in Indonesia. This creates a ripple effect of decreased quality of life, especially amongst low income communities. A regular pack of twenty cigarettes cost under USD $2 in Indonesia, with some brands closer to $1 per pack [34]. It is estimated that an average low-income household spends 15 times more on tobacco than on healthcare and 9 times more on tobacco than on education. Tobacco purchase (12% of total monthly spending) takes precedence over utility bills (water, electricity, phone bills), housing rent, and protein food sources; cigarette spending trails behind only the purchase of rice (22% of

total monthly spending) [17]. According to a joint WHO and World Bank study in 2011, 5% of GDP per capita is spent on buying an average of 100 packs of cigarettes.

As of 2011, approximately 60 million people in Indonesia smoke cigarettes on a daily basis with an average cigarette consumption of 12-13 sticks per day [17, 15]. Smoking initiation typically begins around the ages of 17-18 with almost 13% of smokers beginning before the age of 15 [17]. Gender-wise, more male Indonesians smoke (57%) than females (4%). However, all population sub-groups, particularly children, are affected by the serious and negative impact of second hand smoking (SHS) exposure. Close to 134 million non-smokers in Indonesia are exposed to SHS regularly, which is not surprising given that over 70% of Indonesians live in a household with at least one smoker [3, 17].

Additionally, the lack of smoking bans in public places exposes many individuals to SHS at their place of work and most social and public places. Tobacco-attributed death is estimated to be over 200,000 annually in Indonesia. One in five male deaths and one in ten female deaths are traced back to regular smoking consumption and/or exposure [34]. The main causes of tobacco mortality in Indonesia are cardiovascular diseases, malignant cancers (particularly lung), and chronic, obstructive respiratory illnesses [3]. Approximately, 11 trillion Indonesian rupiah (1.2 billion USD) per year is spent on medical care related to tobacco-attributed diseases [3]. Non-smoking women exposed to SHS at home have an estimated 25% increased risk for lung cancer and an estimated 23% to 25% increased risk for cardiovascular diseases [3].

With nicotine addiction being tied closely to brain chemistry, pharmacotherapy is the most effective method to alleviate withdrawal symptoms, especially for moderate smokers [29]. Several pharmacotherapies for smoking cessation, such as nicotine replacement therapies, varenicline, and bupropion, have been shown to be effective treatments, especially when used in combination [19, 16]. Their availability vary considerably throughout the world [27]. Behavioral therapies are common aids for smoking cessation as well, such as medical counseling (in-person or remote counseling) and support group meetings [13]. Using behavioral therapy in combination with a form of pharmacotherapy protocol has a small but significant impact, increasing success rate by 10-25% compared to pharmacotherapy approaches alone [30].

Given the complexity of nicotine addiction [4], the wide array of treatment approaches [16], and the cost of development of new therapies [22], improvements of currently available treatments is a priority [29, 8]. With medical genetics being applied to smoking cessation, selecting the

most effective treatment protocol for individual smokers is becoming possible [7]. For example, assigning treatment by an individual's nicotine metabolism, has been shown to improve effectiveness of varenicline and nicotine patch [18]. In addition, genetic variants in the CHRNA5 nicotinic receptor has been shown to influence the efficacy of various nicotine replacement therapies [10]. The refinements of treatment protocols based on medical genetics information may improve the likelihood of cessation and minimize side effects [26]. With rapid advancements in technology and decreasing cost in DNA sample collection and processing, many facets of smoking cessation therapy can be improved through genetic-based screening [6].

4.2 Methodology

This market survey was funded by Bina Nusantara University to gather opinions on smoking cessation ability, as well as beliefs on factors that influence cessation success. The 31-question survey was developed to capture information on smokers in three areas: demographics, smoking behavior, and smoking cessation. Current smokers were recruited in 2015 from the greater Jakarta metropolitan area due to its proximity to the Bina Nusantara University campus. Occupations were categorized based on Indonesia's official government identification card; the categories are: civil servant, entrepreneur, educator, and, student. None of the occupations were targeted specifically. There were 111 participants. Preliminary question asking smokers on their willingness to participate in the survey were administered. Upon agreement, a hard-copy of the questionnaire was used as a data collection instrument. The questionnaire was self-administered and completed primarily at the workplace and campuses (for students) with a few completed at public places such as restaurants and food courts. To reduce incompleteness in survey responses, trained interviewers verified missing responses with participants. The data were entered from hard-copy questionnaires into a database that was maintained with mySQL [33]. The descriptive statistics were generated with [R] [23].

4.3 Results

The demographics of the 111 participant smokers are summarized in Table 3. The age range of participants was between 18 and 60 years old (mean of 32 years old). Over 90% of the participants were males. Additionally, most were office workers and students (70%), head of households (47%), and have at least a high school education (95%).

Table 4 summarizes the smoking habits of the participants. 47% smoked between 10 and 20 cigarettes per day while 19% were heavy smokers (over 20 cigarettes per day). We found that 75% of the surveyed participants have attempted to quit smoking at least once. However, less than 20% of those who had attempted were successful in maintaining their

Table 3. Participant Demographics

Average Age (SD) in years	32.4	(11.7)
Gender	Count	(%)
Male	92	(83)
Marital Status		
Married	53	(51)
Occupation		
Entrepreneur	5	(5)
Office Worker	42	(38)
Civil Servant	8	(7)
Student	36	(32)
Educator	18	(16)
Other	2	(2)
Role in Household		
Head of Household	52	(47)
Wife	5	(5)
Child/Dependent	54	(49)
Highest Level of Education		
Elementary School or Less	3	(3)
Middle School	3	(3)
High school	49	(44)
Bachelor's and beyond	56	(51)
Household Size		
2	13	(12)
3	19	(17)
4	40	(37)
5	26	(24)
6	6	(6)
> 6	4	(4)

cessation for over six months; even then, these participants had relapsed and had continued smoking since all participants in the survey were current smokers. 26% of the survey participants reported to have had an illness due to smoking and 19% reported to have seeked medical care related to smoking.

Table 5 further divides the participants based on whether they had attempted smoking cessation or not. For those who had attempted cessation, social environment (36%) and withdrawal symptoms (41%) were the most frequently selected responses for why they believed that their attempts failed. Most quit attempts were unaided (74%) and no participant used pharmacotherapy to attempt quitting. While only 3 (4%) smokers attended a smoking cessation clinic, 11 (14%) received consultation from a doctor, indicating the need of increasing the involvement of primary care providers in smoking cessation. Nearly four-fifths of those who never attempted cessation stated that they wished to quit smoking in the future. 39% felt they could quit with no aid, while 44% admitted that their addiction is the primary reason they have not attempted to quit. Few smokers were aware of screening using medical genetics for cessation in both groups.

Table 6 lists responses on perception on how certain factors affect cessation ability as well as responses to positive and negative reactions to outcomes related to medical genetics and smoking cessation ability. Social environment, exercise, stress level, and satisfaction gained from smoking were the most frequently selected factors in terms of importance on cessation difficulty. Most participants disagreed that they would feel hopeless if they were to find out via medical genetics that cessation would be difficult. Many also responded that they would want to find out more about their children's and family risk of addiction and ability to quit smoking. Overall, 83% of the smokers felt that genetic screening would be useful if it could indicate cessation difficulty and would lead to their consulting their doctors for advice. The majority of responders were interested in knowing the conditions that could be assessed through medical genetics in relation to smoking cessation and smoking-related risks. However, reports on higher likelihood of suffering from cardiac arrest, a known smoking risk factor, would have no effect in motivating lifestyle changes in over three-quarters of the respondents.

Table 4. Smoking-Related Characteristics

Smoking Initiated (Years Ago)	Counts (%)
< 5	24 (22)
5 - 10	28 (26)
11 - 20	31 (28)
21 - 30	14 (13)
> 30	12 (11)
Cigarettes per Day	
< 10	36 (33)
10 - 19	51 (47)
20 - 29	18 (17)
≥ 30	3 (3)
Monthly Cigarette Expense	
< Rp. 100,000	23 (22)
Rp. 100,000 - 300,000	38 (36)
Rp. 300,000 - 500,000	30 (28)
> Rp. 500,000	16 (15)
Illness Due to Smoking	
Has occurred	29 (26)
Have not occurred	82 (74)
Medical care seeked related to smoking	
Yes	21 (19)
No	90 (81)
Cessation Attempted	
Yes	82 (75)
No	28 (25)

Table 5. Questions on Smoking Cessation Attempts

Have attempted to quit (N = 82)		Have not attempted to quit (N = 28)	
Reason for Quit Attempt	Count (%)	Reason for No Quit Attempt	Count (%)
Social or family urging to stop	18 (22)	Feel in control/no need	15 (56)
Health reasons/want to be healthier	52 (63)	Addiction/need to smoke	12 (44)
Financial Reasons	12 (15)	Quit in the Future	
First Quit Attempt (Years)		Yes	22 (79)
< 5	60 (74)	No	6 (21)
6-10	8 (10)	Method if quitting in the future	
> 10	13 (16)	Rehabilitation	0 (0)
Quitting Method		Smoking Cessation Clinic	4 (14)
Rehabilitation	4 (5)	Pharmacotherapy	2 (7)
Smoking Cessation Clinic	3 (4)	Doctor Consultation	7 (25)
Pharmacotherapy	0 (0)	Hypnotherapy	4 (14)
Doctor Consultation	11 (14)	No aid	11 (39)
Hypnotherapy	3 (4)	Involve Medical Treatment	
No aid	59 (74)	Yes	10 (36)
Attempt Outcome		No	18 (64)
Successful	13 (16)	Aware of medical genetics for cessation	
Not Successful	69 (84)	Yes	5 (18)
Reason for Non-Success		No	23 (82)
Social pressure	22 (36)		
Cannot overcome withdrawal symptom	25 (41)		
Relapse for other reason	14 (23)		
Aware of medical genetics for cessation			
Yes	9 (11)		
No	73 (89)		

4.4 Discussion

This market survey has characterized current smokers within an urban city in Indonesia, namely Jakarta. The questions of the survey encompassed participants' cessation attempts and opinions on factors influencing cessation abilities. As is often the caveat with surveys, there were some notable limitations. The sample was generally small due to the labor-intensive process to recruit and minimize missing information from each participant. The survey was also conducted in only in Jakarta due to the location of Bina Nusantara University and convenience. Therefore, generalizability of findings to other areas of Indonesia is limited. In general, participants tended to give more general responses until asked to be more specific. For instance, when responding to how many years ago smoking was initiated, many participants responded with a range rather than a specific number of years.

Despite the seemingly common knowledge of risks associated with smoking, it is still prevalent worldwide and in Indonesia. Smokers tend to underestimate their susceptibility to diseases caused by smoking and are unaware of many facets of how smoking is dangerous [32, 28, 14]. Among the major risk factors of smoking is sudden cardiac arrest [35]. However, we found that few respondents reported that this would motivate them to quit or change their smoking habit if they were informed by medical genetics to be more susceptible towards a cardiac arrest event. Instead between 78-80% responded that it would have no effect on their behavior.

Surveying a target population before marketing medical screening or treatments is important to avoid misinformation and skepticism [31, 5]. Therefore, evaluating the opinions and attitudes of Indonesian smokers towards smoking cessation and genetic screening is essential for commercialization. We showed that assumptions on common knowledge about the dangers of smoking or ways to quit smoking should not be made. In this market survey, it was found that a majority (little over two-thirds) of the participants plan on quitting in the future, mainly without any form of assistance. For those who have attempted cessation, none stated that they used pharmacotherapies. With these responses, it was sensible that very few were also aware of the utility of genetic screening for smoking cessation.

Table 6. Opinions and Attitudes towards Factors that Potentially Influence Smoking Addiction

Factors on Smoking Cessation Ability	Not Important (%)	Important (%)	
Diet	64 (59)	45 (41)	
Exercise	33 (30)	76 (70)	
Social	16 (15)	92 (85)	
Culture	45 (42)	63 (58)	
Alcohol	60 (55)	49 (45)	
Drugs	62 (57)	47 (43)	
Insurance	65 (60)	43 (40)	
Luck	78 (73)	29 (27)	
Stress	21 (19)	88 (81)	
Ethnicity	91 (84)	18 (16)	
Satisfaction from smoking	19 (18)	89 (82)	
Response to Medical Genetics Information on Cessation Difficulty	Agree (%)	Disagree (%)	
No hope of ever quitting	14 (13)	92 (87)	
Determine if offspring has same risk	79 (74)	28 (26)	
Advice other family not to smoke	99 (92)	9 (8)	
Feel that a genetics test was useful	90 (83)	18 (17)	
Consult a doctor for quitting method	90 (83)	18 (17)	
Conditions of Interest for Genetic Report	Yes (%)	No (%)	
Relative high risk for lung cancer, diabetes, cardiac arrest	60 (57)	46 (43)	
The best method for smoking cessation	70 (64)	39 (36)	
Have lower risk for lung cancer despite smoking	64 (59)	44 (41)	
Effect of Knowing Higher Cardiac Arrest Likelihood	More likely (%)	Less likely (%)	Same (%)
Quit smoking	12 (11)	11 (10)	83 (78)
Reduce smoking level	10 (10)	12 (12)	82 (79)
Increase exercise frequency	7 (7)	11 (10)	88 (83)

The use of pharmacotherapy is an effective way to assist smokers during the onset of withdrawal symptoms, especially given that cravings were reported as the main reason for a non-successful cessation attempt and as the reason for never attempting to quit. Therefore, pharmacotherapy can be beneficial for Indonesian smokers, helping them overcome nicotine withdrawal symptoms. In addition to genetics, cessation ability is heavily influenced by environment, lifestyle, and psychological factors [12]. Based on the market survey, Indonesians are more aware of psychosocial and lifestyle factors but not the biological factors that influence smoking cessation. They believed that social environment, exercise, and stress were the most important factors influencing cessation success. As a result, medical consultation and support groups could benefit Indonesian smokers attempting cessation as well.

Several questions were included in the questionnaire to evaluate the reaction towards good and bad news from a genetic test. Based on the survey, Indonesians would reap more benefit than harm from the results of genetic screening stating that they would have difficulties with smoking cessation. Additionally, the majority of respondents stated that they would not feel hopeless upon finding out that they had higher risk for smoking-related diseases. Gauging how smokers believe they could benefit from medical genetics is important [31]. The market survey found that the information that interests Indonesian smokers the most is to determine the best cessation method for them. There was also interest in determining their level of risks for smoking-related diseases.

Beyond benefits for themselves, most of the participants stated that they would like to know if their children had the same predispositions and would encourage their families and friends to quit smoking. Previous studies have shown that smokers having more concern over whom they affect than themselves; particularly when their children are involved [24]. Children as a motivation for cessation could be particularly useful since many Indonesian smokers are heads of households living in homes with 3-5 members.

The market survey discovered that genetic screening would encourage smokers to seek medical help. Market introduction should be done cautiously to avoid the “magic bullet” perception and any other misperception on the capabilities of genetic screening or a particular treatment [21, 25]. The greatest market barrier would be raising awareness and educating the population on treatments available for them; this in turn poses a significant barrier to adding medical genetics to smoking cessation therapy. None of the surveyed participants used pharmacotherapy in their cessation attempts. This was expected given that nicotine replacement

therapies, such as patches and gums, are not available in Indonesia but are available in neighboring countries, such as Singapore [11] while varenicline is available in Indonesia but at a steep price [9].

There is a promising overall attitude towards genetic screening for smoking cessation. Psychotherapeutics combined with counseling could be one avenue to aid in cessation [1], as could providing in-patient treatments for smokers [20]. With advancements, refinements, and a variety of combinatorial aids for pharmacotherapy and behavioral treatment protocols underway, implementation and feasibility testing suitable to Indonesians in various parts of the country could be done in a more structured and informed manner in the future.

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Chapter 5
Indonesian Smokescreen Genotyping Study

5.1 Introduction

Smoking is deeply ingrained in the social culture of a significant portion of the Indonesian population, comprising the fourth highest cigarette consumer in the world [1]. According to a 2011 World Health Organization estimate, 5% of GDP per capita of Indonesia is spent on buying an average of 100 packs of cigarettes and takes precedence over many essential expenditures such as healthcare, education, and housing [2]. On top of the financial cost, it is common knowledge that long-term and second-hand smoking are associated with many complex, terminal diseases [3, 4]. Fortunately, the effects of smoking cessation materialize much more rapidly than the damaging effects of smoking. Additionally, individuals who began smoking at an early age yet stopped smoking before the age of 40 has a 90% higher likelihood of avoiding tobacco-attributed diseases [5]. Therefore, smoking cessation remains to be the most effective way to reduce the impacts of tobacco consumption.

A significant reason behind the low success rates in smoking cessation with- out aid is nicotine's stronghold on brain physiology, which in turn is heavily influenced by an individual's genetic makeup [6, 7]. The CYP2A6 gene plays a major role in the nicotine metabolism pathway [8, 9]. An individual's nicotine metabolism affects the level of circulating and sequestered nicotine and thus nicotine intake [10, 11]. Given the complexity of nicotine addiction [12], the wide array of treatment approaches [13], and the cost of development of new therapies [14], improvements of currently available treatments is a priority [15, 16]. With medical genetics being applied to smoking cessation, selecting the most effective treatment protocol for individual smokers is becoming possible [17]. For example, assigning treatment by an individual's nicotine metabolism, has been shown to improve effectiveness of varenicline and nicotine patch [18]. In addition, genetic variants in the CHRNA5 nicotinic receptor has been shown to influence the efficacy of various nicotine replacement therapies [19]. The refinements of treatment protocols based on medical genetics information may improve the likelihood of cessation and minimize side effects [20]. With rapid advancements in technology and decreasing cost in DNA sample collection and processing, many facets of

smoking cessation therapy can be improved through genetic-based screening [21]. Given the complex biological basis of nicotine addiction, the next logical step would be to begin an initiative dedicated towards resolving issues tied to smoking cessation among the Indonesian population. However, a strategy of this kind has not been introduced in Indonesia.

The United States is attempting to solve these complex, biomedical questions through the Precision Medicine Initiative (PMI) [22]. Early in 2015, President Obama and the National Institutes of Health announced the Precision Medicine Initiative that aims to build a research cohort of one million to use the diversity present within a population as the foundation in designing disease treatment and prevention [23, 24, 25]. Smoking cessation and diseases attributable to smoking were listed among public health issues to be addressed through the PMI approach of curating uniform data from a diverse population [26].

A methodology and tool we have developed, the Smokescreen Genotyping Array is a comprehensive DNA variant assessment tool, with focused content for addiction and pharmacogenetic analysis [27]. We have prototyped the Smokescreen technologies in pilot studies of smokers in the United States [28]. Following our current work in the United States and the nation's PMI initiative, we have applied the same methods to studies of Indonesian smokers. We have designed and piloted a survey for Indonesian smokers and physicians in order to determine the opinions of both groups on medical genetics and smoking cessation treatment. With these existing components and experiences, we are ready to design, build, and implement the Indonesian Biobank that holds clinical and genetic data from Indonesians within the context of nicotine addiction, smoking cessation, and preventable disease (e.g., cancer). This project has established the first biobank in Indonesia focused on major health issues. This project has laid the groundwork for sustainable capabilities in genotyping and computational technologies, databases and sample processing for the Indonesian Biobank, and collaborations with researchers worldwide.

5.2 Study Objectives

The goal of this study was to build upon our current research efforts in nicotine addiction, smoking cessation, and attributable diseases in Indonesia. We aimed to establish the Indonesian Biobank that would be used to study nicotine addiction and cancer based upon a robust and diverse population cohort as well as a detailed and uniform data bank. In the short term, the project begins to determine factors that are crucial in developing

tools and treatment protocols tailored towards Indonesian smokers. In the future, this project will act as a template for precision medicine initiatives focused at understanding and treating other complex health issues facing Indonesians. Specifically, we implemented prototypes of the following:

- Collect DNA samples, phenotypes, and exposure variables on Indonesians based on a cohort selected from epidemiological studies of colorectal cancer that are currently underway at Hasanuddin University.

- Develop capabilities to process DNA and organize clinical and genetic data from multiple studies on nicotine addiction, smoking cessation, and attributable diseases for statistical analyses.

- Perform genome-wide genotyping using the Smokescreen Genotyping Array on collected DNA samples.

- Perform association analyses of known factors that influence nicotine addiction, smoking cessation, and attributable disease (i.e. colorectal cancer).

- Identify domestic and international partners to build and sustain a consortium for genomic research in Indonesia; thereby, allowing for the project to continue to provide value.

5.3 Methodology

Hasanuddin University (UNHAS) has ongoing epidemiology studies involving smokers and cancer. Though a collaboration with UNHAS, we have collected data and samples for colorectal cancer cases and controls. More than 192 cases and controls were selected to be extensively characterized. This sample size was selected based on the number of available unique spaces on the Smokescreen Genotyping Array plates; and sufficient to replicate known associations with moderate-to-large effects. A de-identified identifier was assigned to each individual and used to link phenotype, exposures, DNA samples, and genotyping results. The ethics committee at Hasanuddin University reviewed and approved study protocols.

5.3.1 Phenotypes and Exposures

Previous questionnaires, experts in the field, and the PhenX toolkit was used for standardized measures on phenotypes and exposures [32, 33]. PhenX has suggested collection forms, protocols, and data dictionaries for substance abuse and addiction, including tobacco use and quit attempts. As needed, forms were translated to the Indonesian language by clinical and research staff from both Hasanuddin University and Bina Nusantara University to ensure proper and accurate translations. Measures were nominated by our scientific advisors to increase the number of comparable measures with related studies using the PhenX toolkit [34]. For cancer patients, the questionnaire was expanded with questions from the Cancer Patient Tobacco Use Questionnaire Core and Extension [35].

5.3.2 DNA Samples

DNA samples from blood were collected from cases and controls, both groups included smokers. These samples were stored at UNHAS. In the future, DNA Genotek Oragene (Ottawa, Canada) brand collection may be used to collect salivary samples for DNA extraction [36, 37, 39]. For blood samples, standard protocols for collection and storage were used by UNHAS researchers.

5.3.3 Build Capacity for Genomics Research

Developing tobacco research capacity in Indonesia requires an international team with expertise in clinical research, epidemiology, biostatistics, genetics, and computer science. We have developed prototype IT tools for collection and analysis of genomic research data - both clinical and genetic - in Indonesia.

5.3.4 Physical Infrastructure

Uniform collection and extraction of DNA from blood samples were performed by UNHAS and MRIN clinical staff. The Indonesian Biobank was located in a physically secure area at UNHAS with back-up power and temperature controls. Samples were tracked using their Laboratory Information Management System (LIMS).

5.3.5 Database and Web-based Application

A secure relational database was developed by Bina Nusantara University to store individual-level clinical and genomic data. This database was implemented in PostgreSQL. A web-based application was developed to facilitate secure, multisite entry of data. The application has built-in checks to ensure the quality of data entry into the system. A user

interface was created for this application, enabling user interactions with the system to generate queries and to produce necessary reports and analysis datasets.

5.3.6 Tables, Figures, and Listings (TFLs)

Template TFLs were created to report and summarize the data for measures, DNA samples, and genotyping results. A specification document was created and will serve as a template for additional collections. The document, when finalized, briefly summarize general programming instructions for variables definitions, subgroup definitions, and genotyping classifications. The document contains template outputs categorized by: demographic and study characteristics, DNA, genotyping and quality control, and analysis for smoking and cancer outcomes. Each templates includes the analysis population used, definitions for column variables and totals, analysis variables used, statistics and calculation methods, numeric precision and formatting, and patient subsetting. TFL outputs and reporting were implemented in [R] and can be run on demand.

5.3.7 Sample Genotyping and Quality Control

Extracted DNA from study samples were genotyped on the Smokescreen Genotyping Array [27]. This array is a custom, genome-wide array for research on smoking behavior, addiction, pharmacological treatment, and related disease. It contains 646,247 SNPs and indels for discovery and characterization studies. 1,014 genes relevant to addiction and smoking-related phenotypes were identified by literature search, expert nomination, and biological knowledge-bases. The array contains nearly complete coverage of the chr19q13.2 nicotine-metabolizing enzyme genes, CYP2A6 (612 markers) and CYP2B6 (1,628 markers).

Genotypes and sample-level quality statistics were generated from raw CEL files using the Affymetrix Power Tools software suite and Smokescreen library and annotation files (r2). In brief, samples with a Dish QC value under 0.82, stage I genotyping call rate under 97%, scan failures, positive controls added by the genotyping lab, samples with discordant genotyped vs. reported sex, monozygotic twins, siblings, and replicate samples with the lowest call rate were removed. The Affymetrix SNPolisher software was used to determine the best probeset for each marker and categorize markers based on quality.

A quality controlled dataset was created and linked to the Indonesian Biobank PostgreSQL database developed. Unused DNA may be stored in the Indonesian Biobank for future use.

5.3.8 Genomic Association Analysis

The tables, figures, and listings designed and programmed were performed for each measure. Chi-square tests (for categorical variables) and F-tests (for continuous variables) were used to test for differences in groups. A significance level of 0.05 will be used for these tests.

Generalized linear models were used to evaluate the relationships between smoking and cancer outcomes and genetic and clinical variables. The evaluation includes adjustments for potential confounders, such as age, ancestry, gender, and body mass index. Ancestry was estimated using the principal components analysis of 5,545 ancestry informative markers. Genotypes were coded additively by the number of copies of the reference allele. Given the modest sample size and the large number of variables, the focus is on replicating genetic association cataloged in recent literature and knowledge-bases. These databases include the NHGRI Catalog of Published Genome-Wide Association Studies [39], Gene Ontology [40], and KEGG: Kyoto Encyclopedia of Genes and Genomes [41]. With additional data, we will attempt to extend well fitting statistical models will additional variables to explore associations that may be novel using likelihood ratio tests and penalized regression techniques. Genetic association analyses will be performed in [R] [42].

Preliminary descriptive analyses are reported in Tables 7 and 8 below. Smoking has a huge impact on risk of colorectal cancer in this sample ($p=0.000612$). The risk of this cancer is increased five fold. There are several genetic variants implicated in colorectal cancer.

5.3.9 Future Work

We have formalizing our collaborations through the formation of a consortium of researchers studying nicotine addiction, smoking cessation, and attributable diseases in Indonesia. Updates on this consortium are available on the BDSRC website and includes information on the databases, tools, and resources developed throughout the study to support genomics research. In the future, researchers with data or ongoing studies will be invited to submit a request to join the consortium that will then be reviewed by an advisory committee that will be formed. We will also outreach to other research groups and clinics that currently exist in Indonesia to inform them of the project's efforts.

Table 7. Genotyping Study Participant Characteristics

Description	Overall	Cases	Controls	P-value
Age	54.1 (50.6)	54.1 (13.1)	50.6 (14.4)	0.097
Gender:Female	73 (42.9%)	38 (42.7 %)	35 (43.2%)	0.946
Ethnicity				
Bugis	83 (48.8%)	38 (22.4%)	45 (26.5%)	
Makassar	44 (25.9%)	24 (14.1%)	20 (11.8%)	
Mandar	3 (1.8 %)	2 (1.2%)	1 (0.6 %)	
Non South Sulawesi	13 (7.6 %)	9 (5.3%)	4 (2.4 %)	
Non Sulawesi	8 (4.7 %)	5 (2.9%)	3 (1.8%)	
Toraja	18 (10.6 %)	10 (5.9%)	8 (4.7%)	
BMI	21.1 (7.4)	19.8 (6.0)	22.8 (8.6)	0.020
Smoking				
≥ 100 cigarettes in life	38 (27.0%)	31 (38.8%)	7 (11.5%)	0.000612

A Statistical Analysis Plan (SAP) will be developed in order to formalize how data and results will be summarized across studies in the consortium and how evidence will be translated to precision medicine. The SAP will summarize the background, study design, study conduct and subject characteristics, and statistical methods. A standardized research consent form will be developed and made available to partner studies as part of the SAP.

Additionally, we will pursue the linking of this project to international projects, such as those supported by National Institute on Drug Abuse (NIDA) of the United States, World Health Organization of the United Nations, the Psychiatric Genetics Consortium, and the Precision Medicine Initiative. Results from the study will be presented at an international conference, such as the annual conference held by the Society for Research on Nicotine and Tobacco.

5.4 Scientific Relevance

The first gap this study addresses is the understudied state of the Indonesian population. Indonesia has an ethnically and culturally diverse population that can offer great insights on treatment and disease processes. Conversely, heterogeneity makes it virtually impossible to have a standard protocol of treatment work maximally for every individual patient [22, 23]. The United Kingdom has a well-established national biobank that has facilitated studies ranging from cancer to eye disorders [43, 44]. Scandinavian countries are also in the process of recruiting and collecting

Table 8. Marginal Association Scan Results

Chr	Pos	Allele	P-value	OR
7	19024682	T	2.15E-08	4.4
9	5034454	A	1.22E-06	0.3
19	19425025	C	1.39E-06	0.3
15	25876991	C	1.64E-06	2.9
3	11030852	T	1.77E-06	0.3
5	76050452	G	1.78E-06	0.2
5	176045442	C	2.05E-06	0.3
9	9886403	C	2.25E-06	0.2
7	2953442	A	2.26E-06	0.3
9	5087495	C	2.39E-06	2.9
1	116205021	A	2.74E-06	0.4
7	19021083	A	3.00E-06	2.8
4	515243	G	3.18E-06	0.3
4	516586	A	3.84E-06	0.3
11	133379079	C	4.25E-06	0.3
14	33817553	G	5.23E-06	2.7
17	8523722	A	5.25E-06	0.3
15	94286895	A	5.48E-06	2.8
7	14585513	C	6.04E-06	0.3
16	81937574	T	6.19E-06	0.1
4	175452245	C	6.61E-06	0.2
1	194013039	G	6.83E-06	0.3
4	21348980	C	7.67E-06	4.0
23	83562000	A	8.47E-06	0.2
3	61503354	A	8.65E-06	0.4
2	24680249	G	8.73E-06	2.9
3	72232520	A	9.03E-06	0.2
9	5071049	G	9.42E-06	2.7

data for their national biobanks through universities, hospital networks, and commercial institutions [45, 48]. In Asia, South Korea have begun their biobank establishment [49] while China has multiple biobanking initiatives [50]. Malaysia, India, Singapore, and Thailand have their initiatives as well [51]. With some nations already performing research based on their national biobank and many others in the process of biospecimen curation, it is essential for Indonesia to build its own biobank.

Through the work done in this study, our team will be able to begin identifying and replicating clinical and genetic factors important in an Indonesian population with tobacco related phenotypes. In order to do so, this study ad- dressed another scientific gap that often hampers big-data, biomedical research: the lack of uniform data. Support for standardized data collection of biospecimens and measures across studies [34], including demographics, substance use behaviors, history, and related risk factors, will permit meta-analyses of tests of association with tobacco use dependence and abstinence phenotypes. This biobank design will in turn serve as a template for future biobanking efforts in Indonesia for understanding and treating various complex diseases.

Another gap this study addressed was a lack of collaborative research and data sharing among cancer researchers. The consortium established enables tobacco researchers to share methods and data, increasing sample sizes available to detect important factors. Support for an Indonesian biobank will provide the infrastructure and methods to encourage collaboration among multiple clinical researchers, each of whom will benefit from the availability and cost savings of standardized consents, measures, protocols, and biobanking.

5.5 Broader Impacts

This study impacts biomedicine and the Indonesian population by taking the step towards applying pharmacogenetics to assist treatment-response optimization for smokers and cancer patients. The use of pharmacogenetics is virtually non-existent in Indonesia, including for smoking cessation [52]. Commencing a biobank is a daunting task; thus, a smaller-scale start with a clear and systematic approach based on a major public health issue (e.g. smoking, cancer) proved to be practical. Samples collected from this study achieved our goal to establish the first Indonesian Biobank, supported by refined strategies to manage and maintain it.

Consistent data collection facilitates efforts in expansion, such as the inclusion of more ethnicities and regions, as well as the study of other addictions and smoking-related diseases. The most concrete potential

expansion is through Hasanuddin University's study of colorectal cancer, a disease moderately but significantly associated with smoking [53]. With this study, we can share data and collaborate to design innovative studies.

We have developed an extensive, international network to expand and utilize the Indonesian Biobank.

5.6 Study Sustainability

Uniform and detailed data on clinical and genetic factors related to tobacco use, treatment, and diseases based on a diverse population is extremely valuable to future research, facilitating the study of many facets of biomedicine based on the biodiversity and heterogeneity of the Indonesian population. Additionally, the resources developed in this grant can be used for future investigations of addiction beyond nicotine and colorectal cancer, and potential links to related diseases, such as lung cancer and cardiovascular diseases. The foundation of this study was gathering data using best practices based on our previous research and recommendations from our collaborators and literature. This represents the first biobank in Indonesia with data that can be used for multiple studies.

We have formed and maintained a multitude of international and national collaborations with groups directly and indirectly interested in nicotine addiction and smoking cessation that will play critical roles in propelling the work that will be initiated by this project. In our studies of smokers in the United States, we have published original articles on methodology [27] and a pilot study [28]. Since 2014, we have consistently attended and presented our findings at the annual conference of Society for Research on Nicotine & Tobacco (SRNT), a global organization dedicated exclusively to nicotine and tobacco research with members from forty nations [57]. Additionally, the team is involved in multiple US National Institute on Drug Abuse (NIH/NIDA) contracts and grants (HHSN271201200005C, HHSN271201300004C, and 1R43DA041211-01A1). The software, database, and statistical analysis tools are built in a manner that facilitates both research capacity and training for extensive, future collaborations. Furthermore, we hope to be able to both obtain further support from and stimulate local interest and involvement in order to establish a national biobank for Indonesia that covers multiple geographical regions and ethnicities.

5.7 Innovative Approaches

It is estimated that an annual spending of 11 trillion Indonesian rupiah (an equivalent of 1.2 billion USD) originate from medical care related to tobacco- attributed diseases of the Indonesian population [1, 58]. An typical low-income household is found to spend financially 15 times more on tobacco products than on healthcare and 9 times more than on education [2]. It was also reported that for a significant portion of the low-income household, purchases of tobacco (12% of total monthly spending) often takes precedence over utility bills (water, electricity, phone bills), housing rent, and protein food sources; on average, many household ranks cigarette spending second to only the purchase of rice (22% of total monthly spending) [2].

Big-data medicine is the next big step in biomedical research efforts to solve issues stemming from complex diseases and disorders, such as nicotine addiction. Therefore, it is crucial that Indonesia begins to establish biobanks of clinical data and biospecimens that can serve as the foundation upon which future biomedical projects can be designed and studied. This study begins with colorectal cancer cases and controls, a subset of which are smokers. However, data collection, software design, and statistical methodologies were established with future precision medicine studies in mind. The study was also the first of its kind to layout the platform upon which medical genetics and pharmacogenetics can be utilized, eventually, to select effective treatments and prevention strategies for segments of the Indonesian population.

The use of the Smokescreen Genotyping Array is innovative and aligns with similar ongoing genotyping initiatives by the US NIH/NIDA (Aim 3) [56]. The array is multi-purpose and customizable for studying a variety of diseases and disorders, which allows for a uniform technology that can support the progression of precision medicine work in Indonesia. Building the research capabilities in Indonesia with shared technology and international collaboration allows for data and results to be combined, providing more scientific value.

Chapter 6
Commercialization Roadmap

1. Continue to innovate and educate in high performance computing, genomics, and data science.

    - Through the surveys of physicians and smokers, we learned that there is great interest in medical genetics in Jakarta. However, there is a gap in knowledge on how to use genomic information and the availability of treatments available for smokers wanting to quit. Thus education of physicians and smokers on new medications and screening technologies is needed for commercialization.

    - In the genotyping study we learned that extracting and shipping DNA samples internationally for genotyping is costly and inefficient. Thus the capacity to genotype or sequence DNA samples using high-throughput approaches in Indonesia is very much needed for all human genomic studies throughout the country. This represents a companion commercialization opportunity for BINUS, as there are not yet service providers in Indonesia.

    - Motivated by this research, BINUS has prepared for the data management and analysis requirements for genomic data, by investing in high performance computing and building partnerships with HPC partners such as NVIDIA and AWS. Continuing these activities and training are important for the analysis of genomic data. This represents another companion commercialization opportunity for BINUS, offering HPC and bioinformatics services for other studies.

2. Continue to work with health care partners to collect data and DNA samples. The data from the Smokescreen genotyping project in Indonesia will be extremely useful for research. Discovering and validating biosignatures specific to an Indonesian population for clinical products will require big data. Therefore, an important part of a commercial path is to partner with organizations interested in this research to continue to build the research database. The database itself

could become an extremely valuable asset, it which pharmaceutical and health care networks may license access to high quality data.

3. Use the data to build tools to help smokers quit and treat diseases in Indonesia. The culmination of data collection, high performance computing, and the education path described above is the ability for BINUS to use data science to build multiple health products for Indonesians. This includes tools to help doctors personalize medicine, for the smoker that wants to quit smoker, or the cancer patient to stand the optimal chance of survival. These products could be offered as a service provider where BINUS accepts samples and provides reports to the clinician, licensed to other organizations, or as direct to consumer where the patient plays a more active role in medical decision making based on information provided by us.

Chapter 7
Key Accomplishments

- Through the contributions of BINUS University and their collaborators, we have established the first biobank in Indonesia. While the initial biobank focuses on colorectal cancer and smoking behavior, the requirements for scaling are now known.

- We have developed the IT infrastructure for data collection, management, and analysis of study data. This is composed of a secure relational database, web-based application, and statistical computing support form BINUS, NVIDIA and Amazon Web Services. This system is available for future studies.

- The results of this research has been presented at international meetings and in scientific journals, including:

  - Pardamean B, Budiarto A, Caraka, RE. Indonesian Colorectal Cancer Consortium Data Science System, in preparation.
  - Pardamean CI, Baurley JW, Pardamean B. Pharmacotherapy and medical genetics for smoking cessation in Jakarta. International Journal of Business and Society, under review.
  - Pardamean CI, Baurley JW, Kacamarga MF, Pardamean B, Genetic screening for nicotine dependence and treatment approaches among physicians in Indonesia. International Journal of Pharmaceutical Sciences Review and Research, 43(1), 2017.
  - Pardamean B, Baurley JW, Pardamean CI, Figueiredo JC. Changing colorectal cancer trends in Asians. Int J Colorectal Dis. 2016 Mar 29.
  - Baurley JW, et al. Smokescreen – a unified platform for addiction research. Society for Research on Nicotine and Tobacco Annual meeting. 2016.
  - Baurley JW. et al. Nicotine Metabolism in Multiple Populations. Society for Research on Nicotine and Tobacco Annual meeting. 2015.

  - Baurley JW. et. al. Smokescreen: A Targeted Genotyping Array and Software Application for Nicotine Research. Society for Research on Nicotine and Tobacco Annual meeting. 2014.

- NIDA Genetics Consortium members are aware of the study. There are opportunities for sharing of data and results as the biobank in Indonesia grows.

- We have build strong international collaborations with industry (NVIDIA, Amazon Web Services, AVNET, and Affymetrix); Research institutions (USC, BioRealm, UNHAS, UCLA, Clemson, NTU); and sample processing labs (MRIN, RUCDR).

- We have built BINUS's high performance computing (HPC) capacity for data analysis of genomic data. This includes establishment of the NVIDIA GPU Education Center in 2015, its renewal in 2016, and expansion to an AI R&D Center to be launched summer 2017. In addition, ongoing education and research grants from Amazon Web Services have been awarded for cloud resources.

- The first in Indonesia, an official material transfer agreement (MTA) for DNA transfer to the USA for genotyping was established.

- Sparked collaborations with other research groups across the region (e.g., AsiaGenome, SGInnovate).

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